Metabolic analysis of invitro Antioxidants in Leaves extracts of Ficus *carica.* L

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Abstract

To determine the antioxidant capacity profiles in the leaf extract of Ficus carica leaves. Analysing the antioxidant capacity using several approaches such as TPC, TFC, DPPH, FRAP and GPx analyses confirmations predominantly decrease in fig freeze-dried leaves and seeds. All through, the extraction of fig leaves use of acetone and chloroform confirmations DPPH, FRAP and GPx assesses concomitantly rise which act as strong antioxidant capacity in herbal plants for formulations to ailing the neuropathic and insulin confrontation. Furthermore, analysing the profiles of antioxidants present in the extract can help in understanding the composition and potential therapeutic effects. Metabolic profile of invitro antioxidants results to determine its substrate DPPH is rise especially in leaves of Ficus carica while, comparable with FRAP, GPx activity also parallelly preeminent in leaves extracts which elicits the valuable perceptions into the concealed well-being helps of ficus carica leaves on pharmacological formulations to predict antidiabetic, antifungal, neuropsychological, anti-mutagenic, anti-carcinogenic, and anti-aging responses.

Key words: DPPH, Antioxidant capacity, phenolic, flavonoid content, fig leaves

Introduction

Direction-finding a comprehensive analysis of the invitro antioxidant profile of Ficus carica leaves can provide valuable information on the precise antioxidants existing and their potential health effects (Arvanithi *et al*.,2019). Ficus carica leaves are known to have rich in antioxidant properties and have ailing properties of psychologically and biochemically (Nawaz *et al*., 2020; Sirajo, 2018). The leaves contain many plants related antioxidants such as phenolic compounds, flavonoids, and vitamin C, which possess a pivotal role in cellular levels (Bag *et al*., 2015). These antioxidants support in neutralizing detrimental free radicals in the body, thereby hypothetically present health benefits such as reducing oxidative stress and inflammation (Munira *et al*., 2018; Al-matani *et al*., 2015).

Plant secondary metabolites such as phenolics, flavonoids, coumarins, alkaloids and terpenes which have not only physiological and biological functions in plants but also possesses positive effects for human well-being, which also act as antioxidants (Gil *et al*., 2002; Caliskan and Polat, 2011). Phytoantioxidants metabolites like phenolics, flavonoids, vitamin C, and free radicals scavenge phytobioactive substances such as polyphenols, saponins & beta carotene, consequently impeding the oxidative progression that might be promote to form degenerative ailments (Roleira *et al*., 2015). Phenolic compounds may serve as reducing or donating hydrogen to other compounds, scavenging free radicals, and satiating singlet oxygen (Majid *et al*., 2015). Reactive oxygen species (ROS) causes due to oxidative stress in cell of plants provide defensive role and other harmful molecules also inflated in medicinal herbals (Daniel and Topo., 2012). Existing invitro antioxidants present in the leaves, such as phenolic compounds, ascorbic acid and flavonoids, help to neutralize these free radicals and prevent damage to the cells and tissues (Gliszczynska-Swiglo, 2006; Ghimire *et al*., 2020). This invtro antioxidant activity not only supports the plant's growth and development but also improves its ability to survive ecofriendly strains.

Undeniably, from a biological perception, the antioxidant properties of Ficus carica leaves can also offer possible health aids to humans. Ficus carica leaves or extracts rich in antioxidants might be support in either by oxidizing or reducing oxidative stress in the body, which is associated to innumerable chronic diseases identically cardiovascular disorders, cancer, and neurodegenerative conditions. The presence of invitro antioxidants in Ficus carica leaves such as DPPH, FRAP (Benzie and Szeto., 1999), ascorbic acid and total phenolic content are important to elevate their medicinal properties and make them a vivacious supply

in traditional medicine and natural health therapies (Bursal and Gulcin, 2011). To determine the presence of Phenolics (Gulcin, 2012), alkaloids, anthraquinones, cardiac glycosides, coumarins, flavonoids (Khan *et al*., 2012, saponins, reducing sugar, amino acids, fatty acids, phlobatannins, tannins terpenoids and polyphenols (Sembiring *et al*; 2018; Bursal and Gulcin.,2011; Sahreen *et al*; 2011) in leaves of Ficus carica were subjected to phytochemical screening using acetone, chloroform and 95% methanol extract for further metabolomic profiling in invitro antioxidants. Overall, the invitro antioxidant role in Ficus carica leaves were highly potent for antineurodegenration, antidiabetic and anti-inflammatory activities which indicate a substantial biotic role in plant's defense mechanisms and potential for preventing free radicals scavenging.

Materials and Method

Plant sample collection and extraction

Ficus *carica* plant leaf has collected from the Agriculture Farm of Dhanalakshmi Srinivasan Agriculture College, Perambalur (Affiliated by TNAU, Coimbatore). Medicinal plants have been authenticated as Ficus carica – Moraceae family, traditionally it is known as Atti pazham, Herbarium & Plant Taxonomy Research Unit, PG and Research Department of Botany, St. Joseph's College (Autonomous), Tiruchirappalli, Tamil Nadu, India Voucher No: 2884 following the flora of Tamil Nadu by Henry (1983). Leaves were dried and shredded in to powder using rotary evaporator for metabolic analysis of antioxidants.

Chemicals and equipment's

Plant samples (DSAC, Perambalur), Distilled water (Biochemistry & Biotechnology Laboratory, Dhanalakshmi Srinivasan Agriculture College, Perambalur (Affiliated by TNAU, Coimbatore), Acetone, Chloroform, 95% methanol, DPPH, Ascorbic acid (PSC, Trichy), Rotary evaporator, UV spectrophotometer (Shimadzu, Japan).

Preparation of Ficus *Carica* Leaf extracts

Dried powder of Ficus *Carica* Leaf was exactly weighed to 150 - 300 g and then transferred to a container having 1000 mL of a solvent mixture of ethanol: water (60: 40) and methanol: water (75: 25) separately and then homogenized with a magnetic stirrer. The homogenized blend was extracted for a time period of 72 hours under optimum temperature. The extract was subjected to Whatman No. 1 of filter paper to collect sample and expose to dispersed to

reach a concentration form of the extract used conventionally fraction of organic solvents. The samples collected after fractional process was accomplished for further metabolic study.

Invitro antioxidant analysis of Ficus *Carica* Leaf extracts

Based on the phytochemical analysis for the detection and confirmation of vital plant derived metabolites such as polyphenols, saponin and coumarin compound applied a typical method and for the detection of the rest of many flavonoids and terpenoid species, tannins, and alkaloids (Harborne., 1973).

Total Phenolic Content (TPC):

Add the sample extract with Folin-Ciocalteu reagent and sodium carbonate solution. Incubate the mixture and measure the absorbance at a specific wavelength of 765 nm compare with standard solution of gallic acid. The values are compared with sample and standard solution of TPC measured as mg of gallic acid equivalents per gram of sample (Singleton and Rossi, 1965).

Total Flavonoid Content (TFC):

A standard solution of a flavonoid standard such as quercetin. Add the sample extract with aluminium chloride solution and sodium acetate buffer. Then, incubate the mixture and formation of colour were measured the absorbance at a specific wavelength of 510 nm. TFC tested with standard curve and determined as mg of quercetin equivalents per gram of sample.

DPPH Radical Scavenging Activity

Take 0.1 mM of DPPH solution (3 mL) was treated with 200 µL of sample extract. Keep in a dark room for 30 minutes at ambient temperature. Detection of limit was absorbed were recorded using a spectrophotometer at 517 nm. The results were expressed as ascorbic acid equivalents (AAE) on a DW basis (AAE mg/100 g DW) (Ghimire *et al*., 2020).

Ferric Reducing Antioxidant Power (FRAP):

The plant sample treated with FRAP reagent containing (2,4,6 – Tris (2- Pyridyl)-s-triazine, ferric chloride, and acetate buffer and incubate the samples for 10 mins. Then, quantity the absorbance at a specific wavelength at 593 nm and estimate the FRAP value based on the standard curve is detected (Aguilar *et al*., 1999).

Ascorbic Acid Assay

Weighing 100mg of Ascorbic acid and transferred into 100 ml of standard flask and volume was make up to the mark using solvent system of methanol: water (70:30 v/v) to obtain 1000 µg/ml. From this stock solution, serial dilutions were made to obtain 5, 10, 15, 20, 25µg/mL solutions of Ascorbic acid were done in triplicates and measuring the absorbance at 258 nm (Szeto *et al*., 2002).

Results

The results characteristically confirmation that extracted herbal plants of fig leaves have higher levels of Ascorbic acid (Potent reducing and antioxidant function against bacterial and detoxify reaction in the formation of collagen tissue, bones and skin), Total Antioxidant Capacity (TAC), Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Ferric Reducing Antioxidant Power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, and Vitamin – C (Ascorbic acid) compared to the normal plant. This is due to the concentration of bioactive compounds during the abstraction process, resultant in a more effective antioxidant profile in the herbal extracts. Therefore, extracted herbal plants of fig are prospective to show better antioxidant activity and health benefits compared to human consumption. **Fig.1** shows the phytobioactive molecules such as phenolics, flavonoid saponin, poly phenols & carotenoids naturally ensues as antioxidants and then, they are well known to showed a positive effect on human well-being and can be painstaking for impending uses as plant-based antioxidant machineries in the trade of food and agriculture (Zengin *et al*., 2011; Vallejo *et al*., 2012). The ficus carica known for its high affluence and biodiversity as well as Indian traditional herbal, complementary & traditional with ethnomedicine is also measured as a significant metabolite of mutually innovative drugs and bioactive particles subsequently olden epochs. Future studies will be needed to elucidate more and more medicinal plants and traditional preparations used for dementia, psychosomatic and antidiabetic, cancer therapeutic purposes. Total phenolics and flavonoids content of fig leaf extract were assessed and fond to be more in ethanol and 95% methanol extract which also inhibits cell proliferation, induction of programme cell death and antibacterial effects while compare with acetone and chloroform solvents were identified.

Table.1 Phytochemical composition (mg/100g) of Ficus Carica leaves.

Phytobioactive compounds	Ficus Carica
Phenolics	4.56±0.02*
Flavanoids	6.16±0.02*
Alkaloids	3.45±0.01
Terpenes	2.47±0.02
Carbohydrates	4.23±0.02*
Amino acids	4.12±0.04*
Fatty acids	3.89±0.01
Saponins	4.16±0.01*
Coumarins	2.65±0.02
Anthraquinones	0.93±0.01
Tannins	1.89±0.02
Phlobatannins	1.56±0.02
Cardiac glycoside	2.25±0.02
Starch	2.12±0.02

*Values are mean ± standard deviation of triplicate determination shows statistically significant at $p \geq 0.05$.

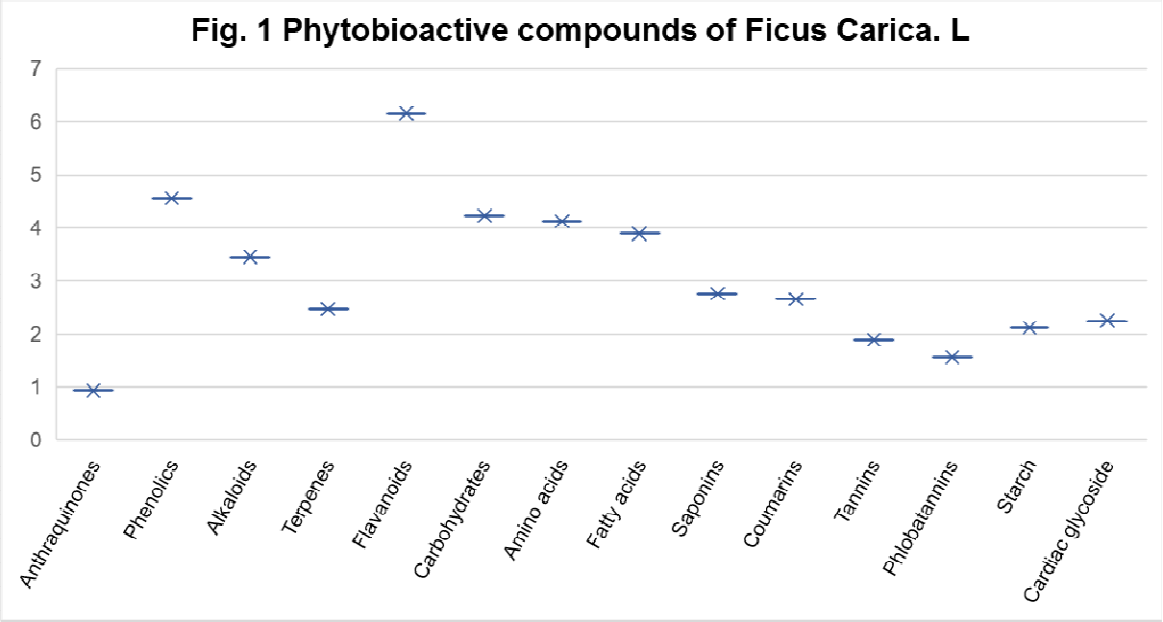
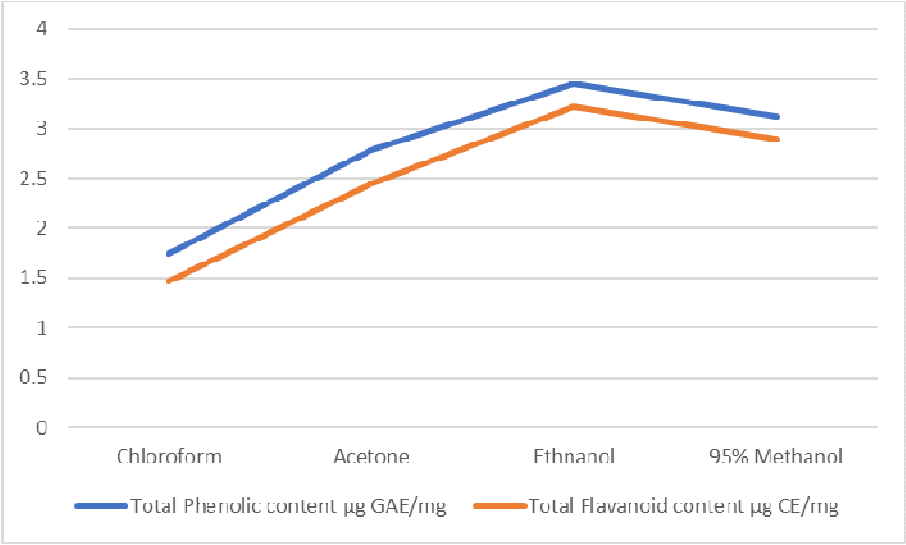
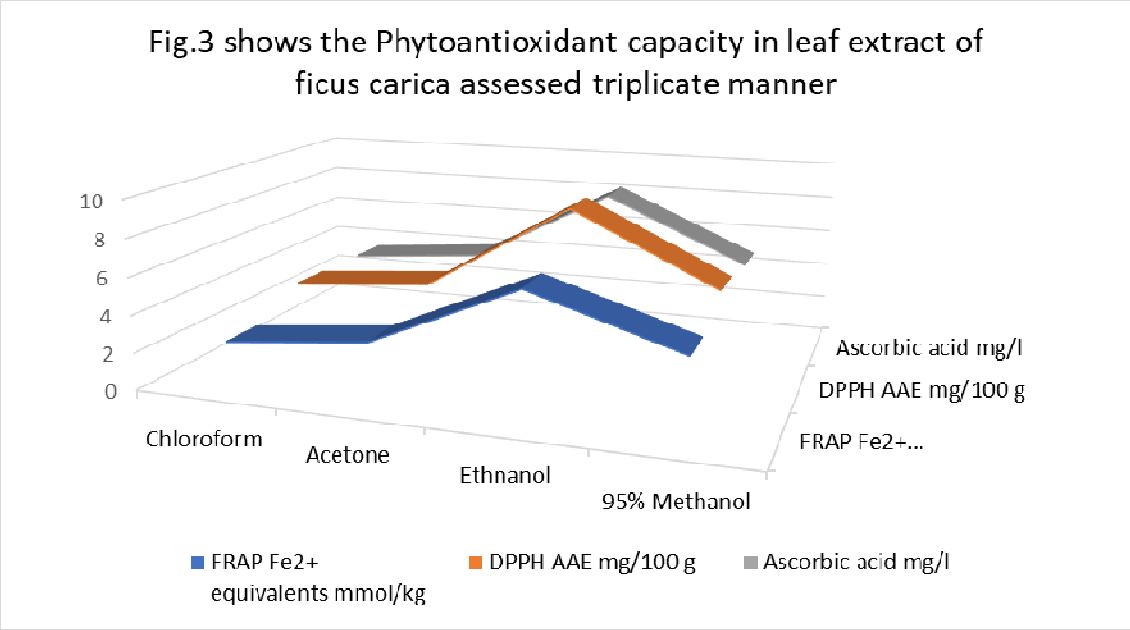


Fig: 2 – Phytometabolic analysis of TPC and TFC in leaf extract of Ficus carica



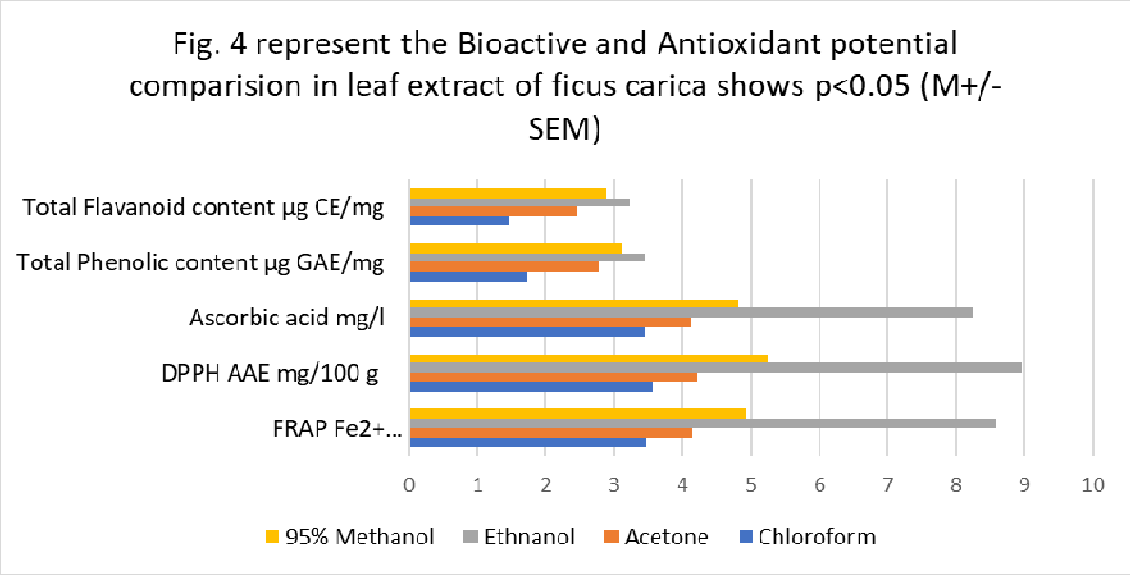
During wide ranging, extracted herbal plants of fig tend to have higher levels of Total Antioxidant Capacity (TAC), Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Ferric Reducing Antioxidant Power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and Vitamin – C (Ascorbic acid) compared to the normal plant. This is because the extraction process concentrates the bioactive compounds present in the plant,

including antioxidants like phenolic compounds and flavonoids **(Fig.2)**. As a result, herbal extracts of fig are often more potent in terms of antioxidant capacity and other related parameters FRAP, DPPH and ascorbic acid possess a substantial rise in Ficus leaf extract for healing human consumption. These higher levels of antioxidants DPPH in herbal extracts can offer improved health aids and pharmacologically therapeutics.



Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) are typically higher in extracted herbal plants of fig compared to the normal plant. This is because the extraction process concentrates these bioactive compounds, resulting in higher levels of phenolic compounds and flavonoids in the herbal extracts. These compounds are known for their antioxidant properties and potential health benefits. Therefore, extracted herbal plants of fig are likely to have higher TPC and TFC levels compared to the normal plant. **Fig. 3** emphasis the results of Ferric Reducing Antioxidant Power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, Vitamin – C (Ascorbic acid), catalase, peroxidase and Glutathione Peroxidase (Gpx) in extracted herbal plants of fig exposed principally rise in ethanol and 95% methanol extraction which produces antioxidant properties related to complementary alternative therapeutics of herbal extracts. Fig leaf extraction done in different solvent possess high concentration in phenolic, flavonoid content, Ferric Reducing Antioxidant Power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity & Vitamin – C (Ascorbic acid) are statistically significant at $p<0.05$ (95% Plant extract possess significant increase in antioxidant capacity), The extraction process

concentrates phytobioactive and metabolic compounds in the herbal extracts, depleted towards the surge in antioxidant potential might leads to degenerative therapy. Therefore, extracted herbal plants of fig are likely to exhibit stronger antioxidant properties as showed by raise in FRAP (85%), DPPH (90% highly significant in fig leaf extract they exert antioxidant effects in cells), and ascorbic acid (82%) they can defences the antinutritional properties in contrast to oxidative damage, they can exert antioxidant effects in the human pancreatic and gastric cells and in other body tissues. Phenolic, flavonoid and saponin content of bioactive compounds are putative in fig leaves might be use as plant extracts to prepare pure compounds as secondary metabolites for the prognosis of cancer, Parkinson, depression, cardiovascular and inflammatory diseases.



Discussion

Fig leaves extract fond to be highly plausible source of total phenolic content and the antioxidant parameter shows a good natural plant antioxidant source in nutrition and pharmacological interferences (Schlesier *et al*; 2002; Sahreen *et al*., 2011)). Phenolic compounds, such as flavonoids and phenolic acids, are known for their strong antioxidant properties (Lako *et al*., 2007). The total phenolic content in fig leaves extract can indicate the presence of these beneficial compounds that contribute to its antioxidant capacity (Sembiring *et al*., 2018). Additionally, measuring the total flavonoid content can also provide insights into the antioxidant potential of fig leaves extract. Both total phenolic and flavonoid contents are commonly used as indicators of antioxidant capacity in plant extracts, including fig leaves (Majid *et al*., 2015).

In leaves extract of fig, the antioxidant capacity can be assessed using various parameters such as Total Phenolic Content (TPC), Total Flavonoid Content (TFC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging is to measure the capacity of antioxidants to scavenge free radicals, and Ferric Reducing Antioxidant Power (FRAP) evaluate the reducing power of antioxidants and investigate the molecular transport ions such as active transport, diffusion, dynamics of proteins, antineoplastic (Roleira *et al*., 2015; Vallejo *et al*; 2012) and medicinal plant based interaction take place between cellular components. These parameters help measure the ability of the fig leaves extract to neutralize free radicals and protect against oxidative stress. High values in these antioxidant capacity parameters indicate a strong antioxidant potential in the fig leaves extract. Conducting these assays can provide valuable insights into the health-promoting properties of fig leaves and their potential benefits in combating oxidative damage.

The antioxidant parameter that typically indicates high antioxidant capacity is the ascorbic acid which has high nutritive value in human. DPPH measures the ability of a substance to neutralize free radicals and prevent oxidative damage (Zengin *et al*., 2011). A higher DPPH, FRAP assay and ascorbic acid value suggests a better antioxidant capacity, signifying that the substance is more active at fighting oxidative stress and protecting cells from damage. Other parameter such as Antioxidant Capacity, phenolics and flavanoid are also consider antioxidant levels, randomly elevated but metabolic substrate like DPPH and FRAP reducing power of antioxidant is alleged as reliable indicator for preventing degenerative diseases.

In conclusion, the Total Antioxidant Capacity (TAC), Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Ferric Reducing Antioxidant Power (FRAP), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays in the leaves of ficus *carica* emphasize the bioactive substances Coumarin, polyphenols, flavanoids and catechins has the property of antioxidant defence (Majid *et al*., 2015). During solvent extract, ethanol shows extremely significant of antioxidants such as DPPH and FRAP compare to methanol and hexane. The TAC measurement reflects the overall antioxidant capacity of fig leaves, while TPC and TFC assessments demonstrate the presence of phenolic compounds and flavonoids specifically in ethanol extraction, which have raise in their antioxidant activities. The FRAP assay indicates the ability of fig leaf antioxidants to reduce ferric ions, contributing to their protective effects against oxidative stress. Furthermore, the DPPH assay shows the efficacy of fig leaf antioxidants in scavenging detrimental free radicals, further emphasizing their role in promoting health and combating oxidative damage. Overall, these bioactive components in

fig leaves work synergistically to provide antioxidant benefits and support for degenerative diseases for aging and lifestyle diseases.

Conflict of Interest

The authors declare that they have no contending interests.

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